

## Effect of nitrogen fertilizer on biomass production and nodulation behavior of *Pongamia pinnata* Pierre seedlings under nursery conditions

S.P. Chaukiyal • Rayees Afzal Mir • T.C. Pokhriyal

Received: 2011-09-05;

Accepted: 2012-10-19

© Northeast Forestry University and Springer-Verlag Berlin Heidelberg 2013

**Abstract:** At the seedling stage, a small amount of N is required to boost growth of leguminous plants. A pot experiment was conducted to observe the effect of N fertilizer on various growth parameters and nodulation behavior of *Pongamia pinnata* under nursery conditions. After the establishment of seedlings, four nitrogen treatments, 0, 40, 80 and 100 kg-ha<sup>-1</sup> N were applied in two equal splits. Monthly observations were taken for the morphological parameters viz. plant height, collar diameter, leaf number, root length, root shoot ratio, nodule number and weight per plant. Maximum plant height was recorded after application of N at 40 kg-ha<sup>-1</sup>. Seasonally, the difference in collar diameter in rainy season was significantly higher than in winter or summer. However, more leaves were produced per plant at N-40 and N-100 treatments in winter and rainy seasons. Higher root length was recorded in rainy season than in winter or summer. Root biomass was higher than for stems or leaves. Seasonal effects of N-80 and N-40 treatments on leaf dry weight were significantly higher than for N-100 or N-0. Stem dry weight was higher at N-40 than at other treatments in winter and summer seasons. Root:shoot ratio was higher throughout winter to early summer. Nodule biomass was 2–3 times higher in rainy season compared to winter or summer. Maximum nodule number and biomass per plant were highest at N-40, followed by N-0, N-80 and N-100 treatments. New nodule formation started from June to the end of September. Maximum biomass per plant was recorded at N-40, followed by N-80, N-100 and N-0. Nitrogen treatment effect and seasonal behaviour interaction were not significant. Significantly higher numbers of nodules per plant were recorded in rainy season followed by summer and winter for all treatments. Higher nitrogen doses suppressed growth while lower doses promoted growth in *Pongamia pinnata*. Therefore, the lower nitrogen dose i.e., N-40 Kg-ha<sup>-1</sup> applied in two equal splits was suitable at the initial nursery stage for the increase in nodulation and biomass production.

**Keywords:** *Pongamia pinnata*; biomass; nitrogen treatments; seasonal behaviour

The online version is available at <http://www.springerlink.com>

S.P. Chaukiyal (✉) • Rayees Afzal Mir • T.C. Pokhriyal  
Plant Physiology, Botany Division, Forest Research Institute, Dehradun, Uttarakhand India.

E-mail: [chaukiyalsp@icfre.org](mailto:chaukiyalsp@icfre.org)

Corresponding editor: Yu Lei

### Introduction

Nitrogen is widely distributed in nature. Weathering of primary rocks to release N is very slow. Consequently, little of the lithosphere nitrogen (N) is supplied to the biosphere. Instead, the major source of biosphere N is the atmosphere, only a very small proportion of plant-available forms (NH<sub>4</sub> and NO<sub>3</sub>) is present in the soil. Due to the very low amounts of available N in soil, farmers and tree growers apply nitrogen fertilizer to increase financial returns. Excessive application of nitrogenous fertilizers is not only undesirable financially, but it also causes adverse effects on our environment and crop quality (NRC 1978; Keeney, 1982). Harper (1974) reported that the addition of soil nitrogen is required for the maximization of biomass at initial nursery stages of growth even for some legume species (Zhang et al. 1990; Becker et al. 1991). *Dalbergia sissoo* (Uniyal 1997) and *Acacia catechu* (Kumar 2005) showed poor responses to higher nitrogen fertilizer doses. Thapliyal (2002) observed increased growth after application of N-40 + P-100 Kg-ha<sup>-1</sup> to *Albizia lebbek*. Sehgal et al. (1992) for *Enterolobium timbouva* and Koul et al. (1995) for *Bauhinia variegata* also reported increases in growth parameters after N application. Therefore, the addition of N fertilizer is invariably needed to maximize crop yields.

Fast growing, multipurpose woody legumes have drawn increasing attention in tropical forestry. Among them *Pongamia pinnata* an indigenous, fast-growing, multipurpose, nitrogen fixer, good coppicer and a suitable tree for waterlogged areas (Misra and Singh 1987 and 1989) and sites irrigated with tannery waste water (Chaturvedi 1986). It is widely distributed throughout India and is now accepted for various agroforestry plantation programmes. It can thrive in areas with annual rainfall from 500 to 2,500 mm (Daniel 2001). Its canopy casts moderate shade (Gilman and Watson 1994; Daniel 2001) and seeds are used in ecofriendly biofuel (Jakowska 2003). However, no information is available describing its N utilization pattern at nursery stage for better growth and development. Our study objective was to describe N fertilizer effects on the plant parts, biomass, and nodu-

lation behaviour of *Pongamia pinnata* at seedling stage.

## Materials and methods

Mature pods of *Pongamia pinnata* were collected from selected healthy trees in the New Forest area, Forest Research Institute, Dehradun, India. The pods were sun dried, and the seeds were separated and sown in germination boxes during April. Fresh soil was collected and transported from the New Forest area to the glass house premises of Plant Physiology Branch, Forest Research Institute, Dehradun. The planting medium of soil, river sand and farm yard manure in 2:1:1 ratio was mixed and sieved. 5 kg of soil mixture filled each pot and 200 pots were prepared and serially numbered for this experiment. After attaining the approximate height of 10–15 cm, seedlings were transplanted into earthen (30 cm diameter) pots in June with a single plant in each pot. These plants were kept outdoors in open space under natural conditions. For the initiation of better growth, a basal fertilizer dose of potash ( $K_2O$ , 58% to 60%) and super phosphate ( $P_2O_5$ , 16%) were applied at  $40\text{ kg}\cdot\text{ha}^{-1}$  and  $80\text{ kg}\cdot\text{ha}^{-1}$ , respectively.

The plants were divided into four groups containing 50 pots each. Four nitrogen (N) treatments were applied: N-40 ( $40\text{ kg N}\cdot\text{ha}^{-1}$ ), N-80 ( $80\text{ kg N}\cdot\text{ha}^{-1}$ ), N-100 ( $100\text{ kg N}\cdot\text{ha}^{-1}$ ) and N-0 control (without nitrogen). Two equal split doses of N fertilizer (i.e., 46% urea) were applied in aqueous solution to treated plants and equal quantity of water was added to control plants (N-0). The first half dose of nitrogen was applied during July and the second in September. Plants were irrigated lightly, immediately after nitrogen application so that the nitrogen was uniformly distributed in the soil and easily available to the plants, and to check evaporation and leaching losses. Watering and other cultural practices were carried out when required.

Morphological observations were recorded at monthly intervals after the plants were well established. Average seasonal activity was calculated for winter (November–February), summer (March–June) and rainy (July–October) by pooling the monthly observations. The uniform plant material was selected by randomized design method. The recorded data were analyzed monthly and seasonally for the whole year (pooled). Fresh weights of individual plant parts were recorded immediately and then kept in the oven at  $80^\circ\text{C}$  for 72 hours for dry weight. Final dry weight was taken when it lost all arable moisture and weights became constant. The number of nodules produced per plant was also recorded before dry weight. All data for each season were analyzed by one-way ANOVA to evaluate differences between treatments. Pooled data for 12 months (i.e., annual data) were analyzed by two ways ANOVA to study the differences between seasons and treatments as well as their interactions.

## Results

An increase in plant height was observed from May to October

in all four treatments (Fig. 1a). Maximum and minimum plant heights were observed in October and April, respectively. Plant heights did not vary by nitrogen treatment during summer or rainy seasons. In annual pooled values, plant heights were significantly higher at N-40 than at N-80 or N-100 treatments (Table 1a). Significantly greater plant heights were recorded during rainy season than in winter or summer. The differences in the plant height due to nitrogen treatment were non-significant.

Collar diameter (mm) followed the trend observed for plant height (Fig. 1b). Maximum values (N-40, 15.68 mm) were recorded in October and minimum (N-0, 7.37 mm) in April. Among different N treatments, N-40 was most effective during winter and summer seasons. Collar diameter did not vary by nitrogen treatment in rainy season. Differences in rainy season collar diameters were only significant compared to winter and summer. In the monthly pooled average values (12 months), N-40 and N-100 treatments yielded significantly higher compared to collar diameters than did N-0 and N-80 treatments (Table 1b).

Maximum numbers of leaves per plant were recorded in October (Fig. 1c), followed by decreases in subsequent months to March. In summer, with increasing ambient temperatures, the soil moisture content decreased and followed along with the commencement of the early summer season and emergence of new leaves started. No significant effects due to nitrogen treatments were observed in the number of leaves during different seasons. However, the maximum and minimum numbers of leaves were recorded for N-40 and N-100 treatments in winter and rainy seasons and for N-100 and N-0 treatments in summer, respectively. Significantly higher numbers of leaves were recorded in rainy season, followed by winter and summer. However, numbers of leaves did not vary significantly by nitrogen treatment (Table 1c).

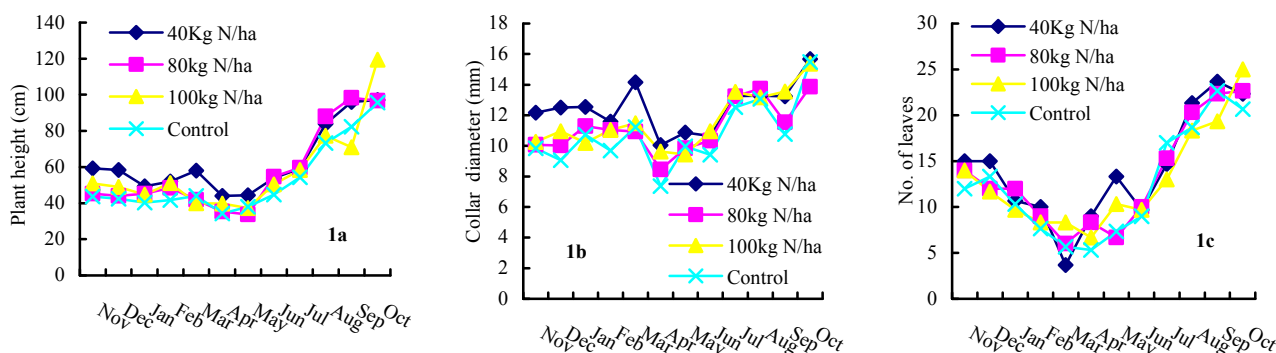
A gradual increase in root length was observed by month (Fig. 2a). Although the nitrogen effects were non-significant in summer and rainy season however, maximum values were observed for N-40 in all seasons. Root length (cm) did not vary by nitrogen treatments. The root length was significantly higher in rainy season compared to winter and summer (Table 1d). Based on seasonal (i.e. winter, summer and rainy season) pooled values, the differences between N-40, N-100 and N-0, N-80 treatments were observed, significantly.

An increase in leaf weight per plant was observed from April to October and was followed by a gradual decrease from November to March (Fig. 2b). No significant differences were observed due to different nitrogen treatments within winter, summer and rainy seasons. Leaf weight was significantly higher in rainy season compared to winter and summer. However, the maximum values were observed for N-40 during winter and summer seasons and for N-80 in rainy season. On the basis of seasonal pooled dry weight values, N-80 and N-40 treatments yielded significantly higher weights than did N-100 or N-0 treatments (Table 2a).

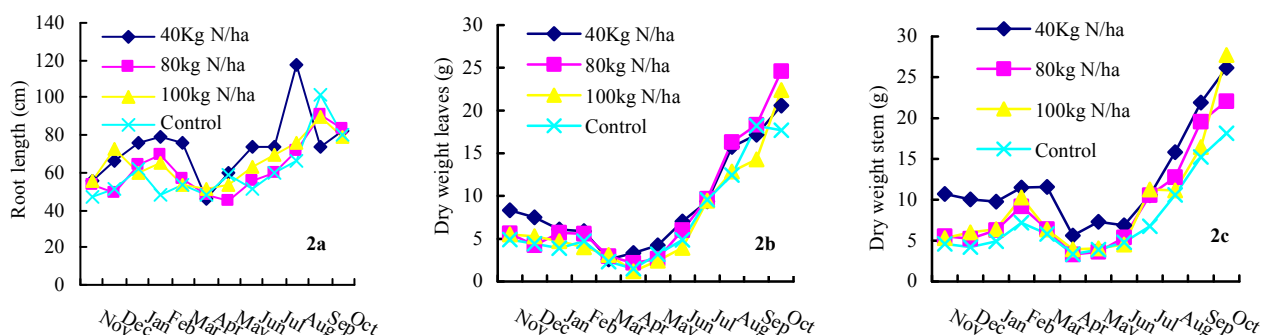
Stem dry weight per plant followed the trend of leaf weight (Fig. 2c). Among N treatments within seasons, N-40 was most effective in winter and summer, whereas, no significant effects were recorded in rainy season. Significantly higher values stem dry

weights were observed recorded in rainy season as compared to than in winter and or summer (Table 2b). On the basis of seasonal

pooled values, N-40 treatment yielded significantly higher stem dry weights than other treatments.



**Fig. 1** Effects of nitrogen treatments on plant height, collar diameter and number of leaves in *Pongamia pinnata* seedlings



**Fig. 2** Effects of nitrogen treatments on plant root length, leaf and stem dry weight in *Pongamia pinnata* seedlings

**Table 1.** Effect of N and seasonal variations on height, collar diameter, number of leaves and root length in *Pongamia pinnata* seedlings

Character	Seasons	Bar diagrams				Level of sig
(a) Height (cm)	Winter	<b>N-40 (54.75)</b>	<b>N-100 (48.96)</b>	<b>N-80 (45.79)</b>	<b>N-0 (38.67)</b>	**
	Summer	N-40 (50.08)	N-100 (41.083)	N-80 (41.38)	N-0 (39.42)	NS
	Rainy	N-80 (85.67)	N-40 (84.08)	N-100 (81.50)	N-0 (76.42)	NS
	Pooled T	N-40 (62.97)	N-80 (57.61)	N-100 (57.43)	N-0 (51.50)	**
	Pooled S	Rainy (81.92)	<b>Winter (47.04)</b>	<b>Summer (43.18)</b>		***
(b) Collar diameter (mm)	Winter	N-40 (12.20)	<b>N-100 (10.61)</b>	<b>N-80 (10.60)</b>	<b>N-0 (9.83)</b>	***
	Summer	<b>N-40 (11.43)</b>	<b>N-100 (10.38)</b>	<b>N-80 (9.77)</b>	<b>N-0 (9.50)</b>	*
	Rainy	N-100 (14.41)	N-40 (13.88)	N-80 (13.10)	N-0 (12.97)	NS
	Pooled T	<b>N-40 (12.51)</b>	<b>N-100 (11.80)</b>	<b>N-80 (11.16)</b>	<b>N-0 (10.76)</b>	***
	Pooled S	Rainy (13.59)	<b>Winter (10.81)</b>	<b>Summer (10.27)</b>		***
(c) No. of Leaves	Winter	N-40 (12.19)	N-80 (11.23)	N-0 (10.54)	N-100 (10.33)	NS
	Summer	N-100 (8.22)	N-40 (7.52)	N-80 (7.02)	N-0 (6.52)	NS
	Rainy	N-40 (23.62)	N-80 (19.45)	N-0 (19.27)	N-100 (18.19)	NS
	Pooled T	N-40 (12.94)	N-100 (11.58)	N-80 (11.54)	N-0 (10.98)	NS
	Pooled S	Rainy (20.03)>	Winter (11.05)>	Summer (7.30)		***
(d) Root length (cm)	Winter	<b>N-40 (66.8)</b>	<b>N-100 (61.98)</b>	<b>N-80 (57.32)</b>	<b>N-0 (51.62)</b>	*
	Summer	N-40 (61.90)	N-100 (54.43)	N-0 (51.79)	N-80 (50.69)	NS
	Rainy	N-40 (84.88)	N-100 (75.21)	N-0 (74.12)	N-80 (74.82)	NS
	Pooled T	<b>N-40 (70.52)</b>	<b>N-100 (63.31)</b>	<b>N-80 (60.13)</b>	<b>N-0 (58.30)</b>	**
	Pooled S	Rainy (77.13)	<b>Winter (59.16)</b>	<b>Summer (54.53)</b>		***

Note: \* = Significant at 5% level; \*\* = Significant at 1% level; \*\*\* = Significant at .1% level; NS = Non-significant, S = Season, T = Treatments, bold text = The treatments under bar are homogenous. The data was transferred to logarithmic scale to stabilize the variance of the treatments.

**Table 2. Effects of N and seasonal variation on plant part biomass with nodule number and nodule biomass in *Pongamia pinnata* seedlings**

Characters	Season	Bar Diagrams				Level of sig
(a)	Winter	N-40 (6.39)	N-80 (4.94)	N-100 (4.80)	N-0 (4.34)	NS
D.Wt	Summer	N-40 (3.80)	N-80 (3.40)	N-0 (2.69)	N-100 (2.37)	NS
leaves	Rainy	N-80 (16.10)	N-40 (14.96)	N-100 (13.73)	N-0 (13.67)	NS
	Pooled T	<b>N-40 (7.13)</b>	<b>N-80 (6.47)</b>	<b>N-0 (5.43)</b>	<b>N-100 (5.39)</b>	*
	Pooled S	Rainy (14.58) >	Winter (5.06) >	Summer (3.02)		***
(b)	Winter	N-40 (11.58)	<b>N-100 (6.83)</b>	<b>N-80 (6.03)</b>	<b>N-0 (5.01)</b>	***
D.Wt	Summer	N-40 (7.31)	<b>N-100 (4.50)</b>	<b>N-80 (4.18)</b>	<b>N-0 (4.16)</b>	***
Stem	Rainy	N-40 (17.14)	N-80 (15.30)	N-100 (15.19)	N-0 (11.71)	NS
	Pooled T	N-40 (11.32)	<b>N-100 (7.76)</b>	<b>N-80 (7.28)</b>	<b>N-0 (6.24)</b>	***
	Pooled S	Rainy (14.7)	<b>Winter (6.99)</b>	<b>Summer (4.89)</b>		***
(c)	Winter	N-40 (17.23)	<b>N-100 (11.68)</b>	<b>N-80 (10.99)</b>	<b>N-0 (9.13)</b>	*
D.Wt	Summer	N-40 (11.63)	<b>N-100 (7.51)</b>	<b>N-0 (7.32)</b>	<b>N-80 (6.76)</b>	*
Root	Rainy	N-40 (25.61)	N-80 (22.34)	N-100 (19.97)	N-0 (19.07)	NS
	Pooled T	N-40 (17.24)	<b>N-100 (12.05)</b>	<b>N-80 (11.88)</b>	<b>N-0 (10.84)</b>	***
	Pooled S	Rainy (21.61) >	Winter (11.92) >	Summer (8.11)		***
(d)	Winter	N-40 (33.44)	<b>N-100 (22.83)</b>	<b>N-80 (22.34)</b>	<b>N-0 (18.79)</b>	**
D.Wt	Summer	N-40 (23.57)	<b>N-0 (16.83)</b>	<b>N-100 (14.83)</b>	<b>N-80 (14.19)</b>	*
Per plant	Rainy	N-40 (59.09)	N-80 (54.06)	N-100 (49.37)	N-0 (44.76)	NS
	Pooled T	N-40 (35.98)	<b>N-100 (26.67)</b>	<b>N-80 (26.17)</b>	<b>N-0 (24.19)</b>	**
	Pooled S	Rainy (51.54) >	Winter (23.80) >	Summer (17.74)		***
(e)	Winter	N-40 (3.33)	<b>N-100 (2.91)</b>	<b>N-80 (2.85)</b>	<b>N-0 (2.66)</b>	**
Shoot	Summer	N-40 (2.90)	<b>N-80 (2.53)</b>	<b>N-0 (2.50)</b>	<b>N-100 (2.34)</b>	NS
D.Wt	Rainy	N-80 (4.66)	N-40 (4.35)	N-100 (4.09)	N-0 (4.04)	NS
	Pooled T	N-40 (17.64)	<b>N-80 (14.87)</b>	<b>N-100 (12.84)</b>	<b>N-0 (12.52)</b>	**
	Pooled S	Rainy (28.41)	<b>Winter (11.89)</b>	<b>Summer (8.71)</b>		***
(f)	Winter	N-40 (1.09)	N-80 (1.01)	N-0 (1.01)	N-100 (0.97)	NS
Root shoot ratio	Summer	N-100 (1.15)	N-40 (1.06)	N-0 (1.00)	N-80 (0.86)	NS
	Rainy	N-40 (0.80)	N-0 (0.76)	N-100 (0.70)	N-80 (0.67)	NS
	Pooled T	N-40 (0.99)	N-100 (0.94)	N-0 (0.92)	N-80 (0.84)	NS
	Pooled S	Winter (1.02)	Summer (1.01)	Rainy (0.73)		NS
(g)	Winter	N-40 (1.78)	N-80 (1.58)	N-100 (1.53)	N-0 (1.43)	Ns
D.Wt nodule	Summer	N-0 (1.38)	N-40 (1.32)	N-80 (1.15)	N-100 (1.14)	NS
	Rainy	N-0 (2.50)	N-80 (2.46)	N-40 (2.45)	N-100 (2.08)	NS
	Pooled T	N-40 (1.79)	N-0 (1.70)	N-80 (1.64)	N-100 (1.54)	NS
	Pooled S	Rainy (2.37) >	Winter (1.57) >	Summer (1.24)		***
(h)	Winter	N-40 (12)	N-80 (11)	N-100 (10)	N-0 (9)	NS
nodule number	Summer	N-40 (7)	N-0 (7)	N-80 (7)	N-100 (6)	NS
	Rainy	N-40 (22)	N-0 (18)	N-80 (17)	N-100 (17)	NS
	Pooled T	N-40 (13.79)	<b>N-80 (11.56)</b>	<b>N-0 (11.54)</b>	<b>N-100 (11.12)</b>	***
	Pooled S	Rainy (19)	Winter (11)	Summer (7)		*

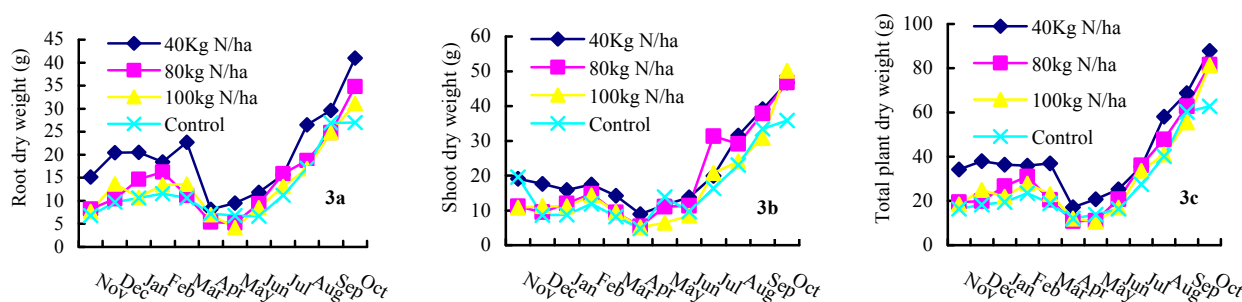
Note \*= Significant at 5% level; \*\* = Significant at 1% level; \*\*\* = Significant at .1% level; NS = Non-significant, S = Season, T = Treatments, bold text = The treatments under bar are homogenous. The dry weight is given in gms. The data was transferred to logarithmic scale to stabilize the variance of the treatments

Root dry weight per plant followed the trend of leaf and stem dry weights (Fig. 3a). Maximum root dry weights were observed for N-40 compared to other treatments in all seasons. Nitrogen treatment effects were non-significant among themselves in rainy season (Table 2c). In annual pooled values, N-40 treatment yielded significantly higher compared to root dry weights than did N-80, N-100 or N-0 treatments. Significantly higher biomass per plant was obtained in rainy season, followed by winter and summer.

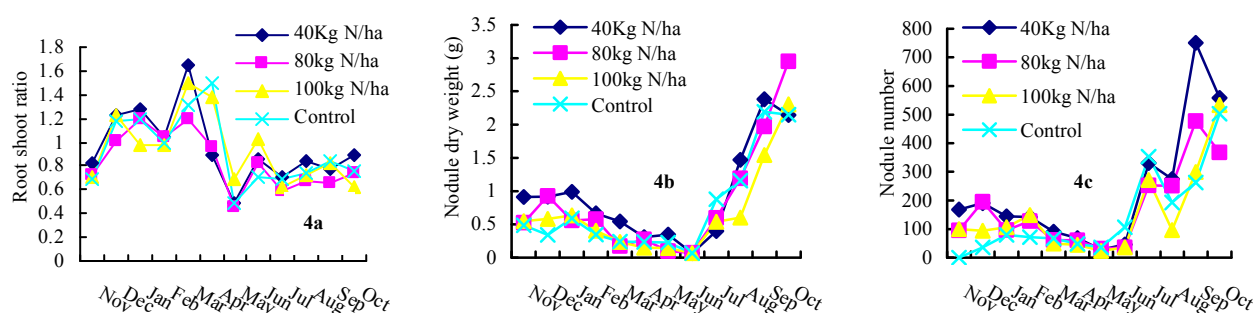
Shoot dry weight followed the trend observed for leaf, stem and root dry weight (Fig. 3b). From winter to early summer months, shoot biomass was nearly constant but from late summer it increased gradually up to rainy season. Minimum and maximum shoot dry weights were observed in April and October, respectively. The season wise analysis indicated that the variation between the treatments during all three seasons was highly significant and the yearly pooled data analysis also indicated almost similar results (Table 2e). The lowest N (N-40) dose was more effective and

yielded better performance, and rainy season produced more shoot

biomass than did summer or winter.



**Fig. 3** Effects of nitrogen treatments on root, shoot and total dry weight per plant in *Pongamia pinnata* seedlings



**Fig. 4** Effects of nitrogen treatments on root shoot ratio, nodule dry weight and nodule number per plant in *Pongamia pinnata* seedlings

Total biomass (leaf + stem + root) followed the trend of individual plant parts (Fig. 3c). Increased total plant weight was recorded from April to the end of rainy season. Significantly, higher biomass per plant was observed in recorded for N-40 treatment during all three seasons. N-treatment effects varied significantly during winter and summer, but not during rainy season. In the pooled average values, N-40 treatment yielded significantly higher dry weights compared to other treatments. Significantly, higher values for dry weights were recorded in rainy season than in winter or summer (Table 2d).

The root:shoot ratio increased during winter to early summer (March/April). Maximum values were observed in March (N-40) and minimum in May (N-80) due to different nitrogen treatments. The ratio remained low (less than 1.00) throughout rainy season as well as for different nitrogen treatments (Fig. 4a). Root:shoot ratios did not vary by treatment within seasons. The year wise analysis also revealed that the treatments did not differ significantly by season (Table 2f).

Dry weights of nodules progressively increased from July to October followed by a gradual decrease (Fig. 4b). Nodule biomass increased by 2-3 times in rainy season than in winter and summer. The lowest nodule dry weight was recorded in June. Nodule dry weights did not vary significantly by treatment but 40 kg-ha<sup>-1</sup> N yielded higher biomass than other doses. On the basis of annual average values, the lowest nitrogen dose (N-40) showed better performance followed by N-0, N-80 and N-100. However, in most cases higher doses suppressed nodule development and lower doses favored greater dry weights. Treatment effects on dry weight

varied significantly during summer between N-40, N-0 and N-100, N-80 (Table 2g) but no significant differences were observed in nodule dry weight during different seasons (Table 2g). Nodule dry weight increased significantly during rainy season followed by winter and summer. Rainy season seemed to be more favorable for nodulation than winter or summer.

An increase in nodule number per plant was observed from June to October followed by a sharp decrease in winter (Fig. 4c). During summer, fewest nodules were formed. Maximum nodules were observed in September and minimum in May. The formation of new nodules started from June and continuously increased to the end of rainy season. Nitrogen treatments and seasonal variation effect on nodule formation was non-significant. However, N-40 treatment showed the best performance throughout the seasons (Table 2h). On the basis of annual averages, significantly higher values were observed for N-40 compared to N-80, N-0 or N-100. The number of nodules per plant was significantly higher during rainy season followed by winter and summer.

## Conclusion

Growth of plant parts such as leaf, stem and root increased with plant age. The maximum increment was observed in rainy season (July–October). *Pongamia pinnata* being deciduous, the leaves were shed during March and April. Hence, the physiological and biochemical activities associated with the growth phenomenon

remained idle during this period. On the other hand, plants suffered harsh environmental conditions, i.e., increased solar radiation and atmospheric temperature, and decreased soil moisture and relative humidity that had a negative effect on growth at this stage.

Among different plant parts, root biomass was always higher (approximately 50%) than stem biomass and leaf biomass was lowest over all N treatments and seasons. Vasileva et al. (2011) reported that the application of mineral N exerted a stronger influence on the quantity of dry root biomass. Highest root biomass was recorded in September for *Lespedeza* by Xu et al. (2007), also supporting our results. Fluctuation of below ground biomass production within a growth season is a common phenomenon which is well documented by Shackleton et al., (1998) and Synman (2005). The root:shoot ratio was higher throughout winter to early summer and then followed by a decrease, which remained almost constant until the onset of rainy season. These changes might be due to the plants shedding their leaves during this period. Low root:shoot ratios during the active growing season indicates high water use efficiency (WUE) of this species. Somewhat similar results were obtained by Xu et al. (2007) for some legume herbs. Ryle et al. (1978) reported that the nodulated leguminous plants always have higher root:shoot ratios, because their roots are metabolically more active compared to non-nodulated roots and act as a strong carbon sink and exhibit a wider range of metabolic process. Xu et al. (2007) also reported for sainfoin and *Lespedeza davurica* the more efficient downwards carbon sink. Low root:shoot ratios were recorded as plants aged. This is also justified by the fact that the root:shoot and photosynthetic:non-photosynthetic biomass ratios decreased with age (Shah et al. 1998).

The N effect was not significant between treatments and the maximum biomass values were recorded for N–40 followed by N–80, N–100 and N–0. Similar trends were observed in the biomass of individual plant parts. It was expected that *Pongamia pinnata* would be less responsive to higher nitrogenous fertilizer doses. Several studies showed that a wide variety of plant species are able to take up organic N compounds especially under low N conditions (Schimel and Chapin 1996; Nasholm et al. 1998; Nasholm et al. 2000; Hodge et al. 2000; Harrison et al. 2008; Nasholm et al. 2009). Similarly, an adverse effect of fertilizer N has already been explained elsewhere in *Dalbergia sissoo* by Hussain et al. (1990); Uniyal and Pokhriyal (2000); Kumar (2005); Indieka and Odee (2005). Ayneband et al. (2012) also reported that higher concentration of soil N decreased and lower concentration increased the biomass in *Triticum aestivum*. Ayneband et al. (2010) explained that N fertilizer not only promoted mineral elements in the soil, but is usually essential for growth enhancement and maximizes yield.

Maximum increase in nodule weight was observed at N–40 and thereafter it decreased with increase in the nitrogen application (Fig. 4b). Rainy season was observed to produce maximum nodule numbers compared to winter and summer in all N treatments. Maximum nodule numbers were produced during rainy and winter seasons at 40 kg·ha<sup>-1</sup> N followed by 80 kg·ha<sup>-1</sup> N, 100 kg·ha<sup>-1</sup> N, and N–0, while higher doses suppressed nodulation (Hussain et al., 1990) in other species. Similar results were reported for *Phaseolus vulgaris* (Shamseldin and Moawad 2010, Danso et al. 1990) and for other species by Borin et al. (1992), Carr et al. (2000), Cheema

and Ahmad (2000), Vasileva and Kostov (2002), Vessey (2002), Anon (2004), Achakzai (2007) and Vasileva et al. (2011).

Nitrogen supply at early stages of growth can result in rapid initial growth and after stabilization, plants were able to undertake N fixation at higher rates. Williams & Haynes (1995) explained that at initial stage a crop has limited demand for N but the requirement increases rapidly as the plant grows. In many situations soil N cannot match plant requirements during this phase and fertilizer may be required to maintain maximum growth rates. Reduction in nodule growth due to higher N doses in *Pongamia pinnata* might be due to inhibition in root hair infection or lectin synthesis and thus prevents the association between rhizobia and root hairs as earlier explained by Johnsen and Bongarten (1991), Sawhney et al. (1991) and Sehgal et al. (1992). In general, decrease in N uptake in plants treated with higher N doses is attributed to toxicity of plants leading to interference of the regulatory plant root mechanisms responsible for nutrient uptake (Elmer 1999). N uptake in plants solely dependent on N<sub>2</sub> fixation compared very poorly with those supplied with optimal N treatments thus emphasizing the need to supply a limited amount of N (starter N) to stimulate early and effective symbiosis (Indieka and Odee 2005).

In *Pongamia pinnata*, the initiation of fresh nodules takes place during summer when plants face adverse conditions resulting in a marked suppression in the nitrogenase activity during this period (Chaukiyal et al. 2000). After the completion of the stress period and with the onset of the rainy season, the atmospheric conditions become more favorable to overall growth and other metabolic activities. Almost similar results were earlier reported in for *Dalbergia sissoo* and *Acacia nilotica* by Pokhriyal et al. (1990 and 1991). In our study, N treatment effect and seasonal behaviour interaction were non-significant, but significantly, higher nodule number more nodules per plant was recorded in rainy season, followed by winter and summer.

Greater nitrogen applications were effective in stimulating growth and nodulation in *Pongamia pinnata* seedlings. But excessive nitrogen fertilizer application should be avoided for leguminous tree species such as *Pongamia pinnata* or should be applied in splits after assessing the requirement to maintain a consistent availability of nitrogen to maintain soil fertility. On the basis of these studies the best results were recorded for N–40 treatment. Initially, low nitrogen starter doses will be helpful in boosting seedling growth, whereas excessive nitrogen fertilizer application will inhibit the process of biological nitrogen fixation, on one hand and deplete it through volatilization, leaching, polluting atmosphere and rhizosphere respectively on the other. Plant growth can be maintained by adopting suitable agronomic management practices, so that a demand and supply is well maintained and plants can fully utilize the fertilizer applied. It can draw the benefits of atmospheric nitrogen fixation simultaneously by establishing strong host-parasite relationships. The perfection of these techniques will ultimately enable the plantation to be raised under various afforestation programmes and to increase the productivity depending on the agro-climatic sites of the country.

## References

- Achakzai AKK. 2007. Effect of various level of nitrogen fertilizer on nodulation of pea cultivars. *Pakistan Journal of Botany*, **39**(5): 1673–1680.
- Anonymous. 2004. Field pea-production and management. In: *Crops January*. Manitoba: Manitoba Agriculture, Food and Rural Initiatives.
- Ayneband A, Gerami F, Fateh E. 2012. Effect of different green manure crops and nitrogen levels on biomass production efficiency and nitrogen concentration in wheat (*Triticum aestivum* L.) and soil. *Advances in Environmental Biology*, **6**(1): 362–367.
- Ayneband A, Tehrani M, Nabati DA. 2010. Residue management, and N-splitting methods effects on yield, biological and chemical characters of canola ecosystem. *Journal of Food, Agriculture and Environment*, **8**(2): 317–324.
- Becker M, Diekamnn KH, Ladha JK, De Datta SK, Ottow JCG. 1991. Effect of NPK on growth and nitrogen fixation of *Sesbania rostrata* as green manure for low land rice (*Oryza sativa* L.). *Plant and Soil*, **132**(1): 149–158.
- Borin MS, Nosca BG, Giardini L. 1992. Effect of nitrogen fixation and soil type on nitrogen nutrition in soybean. In: *Proceeding of Second Congress Soc. Agronomy*. Warwick University, pp. 23–28.
- Carr PM, Henson RA, McKay KR. 2000. Inoculation and fertilization of field pea. Annual Report Agronomy Section, Dickinson Research Extension Center 1089 State Avenue Dickinson, ND 58601.
- Chaturvedi AN. 1986. Trees and shrubs for control of tannery waste water in India. *Environmental Conservation*, **13**(2): 164–165.
- Chaukiyal SP, Sheel SK, Pokhriyal TC. 2000. Effects of seasonal variation and nitrogen treatments on nodulation and nitrogen fixation behavior in *Pongamia pinnata*. *Journal of Tropical Forest Science*, **12**(2): 357–368.
- Cheema ZA, Ahmad A. 2000. Effects of urea on the nitrogen fixing capacity and growth of grain legumes. *International Journal of Agriculture and Biology*, **2**(4): 388–394.
- Daniel NJ. 2001. *Pongamia pinnata*: a nitrogen fixing-tree for oilseed. In: Roshetko, J.M. (ed), *Agroforestry Species and Technologies*: a compilation of the highlights and fact sheets published by NFTA and FACT Net 1985–1999. Taiwan Forestry Research Institute and Council of Agriculture. Taiwan, Republic of China; Winrock International, Morrilton, Arkansas, USA.
- Danso SKA, Japata F, Awonaike KO. 1990. Effect of post-emergence, supplemental inoculation on nodulation and symbiotic performance of soyabean (*Glycine max* L. Merrill) at three levels of soil nitrogen. *Applied Environmental Microbiology*, **56**: 1793–1798.
- Elmer WH. 1999. Influence of ammonium sulfate and rotational crops on strawberry black rot. *Plant Disease*, **83**: 119–123.
- Gilman EF, Watson DG. 1994. *Pongamia pinnata* Pongam. Fact Net Sheet ST-498. A series of the environmental Horticulture Department, Florida Cooperative Extension Service, Institute of food and Agricultural Sciences, University of Florida, USA.
- Harper JE. 1974. Soil and symbiotic nitrogen requirements for optimum soybean production. *Crop Science*, **14**: 255–260.
- Harrison KA, Bol R, Bardgett RD. 2008. Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? *Soil Biology and Biochemistry*, **40**: 228–237.
- Hirel B, Tetu T, Lea PJ, Dubois F. 2011. Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability*, **3**: 1452–1485.
- Hodge A, Robinson D, Fitter A. 2000. Are microorganisms more effective than plants at competing for nitrogen? *Trends in Plant Science*, **5**(7): 304–308.
- Hussain A, Sharar M, Slam F. 1990. Growth, nodulation, nutrient uptake and nitrogen fixation of *Dalbergia sissoo* as affected by different levels of nitrogen. *Nitrogen Fixing Tree Research Report*, **8**: 127–129.
- Indieka SA, Odee DW. 2005. Nodulation and growth response of *Sesbania sesban* (L.) Merr. to increasing nitrogen (ammonium) supply under glasshouse conditions. *African Journal of Biotechnology*, **4**(1): 57–60.
- Jakowska S. 2003. *Pongamia pinnata*: Another miracle tree?, *NFT News*, **6**(1): 6.
- Johnsen KH, Bongarten BC. 1991. Allometry of acetylene-reduction and nodule growth of *Robinia pseudoacacia* families subjected to varied root zone nitrate concentrations. *Tree Physiology*, **9**: 507–522.
- Keeney DR. 1982. Nitrogen management for maximum efficiency and minimum pollution. In: Stevenson FJ (ed), *Nitrogen in Agricultural Soils*. Madison Wisconsin: American Society of Agronomy.
- Koul VK, Bhardwaj SD, Kushal AN. 1995. Effect of N and P application on nutrient uptake and biomass production in *Bauhinia variegata* Linn. seedlings. *Indian Forester*, **121**(1): 14–22.
- Kumar P. 2005. Nitrogen fixation and assimilation behaviour in relation to seasonal variation in *Acacia catechu*. Thesis submitted to F.R.I. Deemed University, Dehradun, pp. 135.
- Misra CM, Singh SL. 1987. Ecological evaluation of certain leguminous trees for agroforestry. *Nitrogen Fixing Tree Research Report* 5, p. 5.
- Misra CM, Singh SL. 1989. Coppice regeneration of *Cassia siamea* and *Pongamia pinnata*, *Nitrogen Fixing Tree Research Report* 7, p. 4.
- Nashlorn T, Ekblad A, Nordin R, Giesler M, Hogberg M, Hogberg P. 1998. Boreal forest plants take up organic nitrogen. *Nature*, **392**: 914–916.
- Nasholm T, Kielland K, Ganeteg U. 2009. Uptake of organic nitrogen by plants. *New Phytologist*, **182**: 31–48.
- Nashlorn T, Huss-Danell K, Hogber GP. 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. *Ecology*, **81**: 1155–1161.
- National Research Council (NRC). 1978. *Nitrates, an Environmental Assessment: a report*. Washington DC: National Academy of Science, p.723.
- Pokhriyal TC, Chaukiyal SP, Negi DS, Gupta BB. 1991. Effect of nitrogen treatments on *in-vivo* nitrate reductase activity in *Enterolobium timbouva*. *Indian Forester*, **117**(1): 72–74.
- Pokhriyal TC, Negi DS, Chaukiyal SP, Bhandari HCS, Gupta BB. 1990. Changes in nitrogenase and nitrate reductase activity in *Acacia catechu*. *Nitrogen Fixing Tree Research Report* 8, pp. 108–110.
- Ryle GJA, Powell CE, Gordon AJ. 1978. Effect of source of nitrogen on the growth of fiskeby soybean: The carbon economy of whole plants. *Annals of Botany*, **42**: 637–648.
- Sawhney V, Singh R, Sawhney SK. 1991. Nitrate reduction and its effect on nitrogen fixation in sliced nodules of pigeon pea. *Phytochemistry*, **30**(1): 51–55.
- Schimel JP, Chapin FS. 1996. Tundra plant uptake of amino acid and  $\text{NH}_4^+$  nitrogen *in situ*: Plants compete well for amino acid N. *Ecology*, **77**: 2142–2147.
- Sehgal JS, Shanker V, Sehgal HS, Pokhriyal TC. 1992. Effect of nitrogen fertilizers on the nitrogenase activity and rhizosphere microflora in *Enterolobium timbouva* Mart. *Indian Forester*, **118**(3): 218–226.
- Shackleton CM, McKenzie B, Granger JE. 1998. Seasonal changes in root biomass, root/shoot ratios and turnover in two coastal grassland communities in Transkei. *South African Journal of Botany*, **54**: 465–471.
- Shah VK, Bana OPS, Singh V. 1998. Biomass production and its allocation in components of young *Robinia pseudoacacia*. *Tropical Ecology*, **39**(1): 1–10.

- 125–131.
- Shamseldin A, Moawad H. 2010. Inhibition of nitrogenase enzyme and completely suppression of nodulation in common bean (*Phaseolus vulgaris* L.) at high level of available nitrogen. *American-Eurasian Journal of Agriculture and Environmental Science*, **7**(1): 75–79.
- Synman HA. 2005. Rangeland degradation in a semiarid South Africa. I: Influence on seasonal root distribution, root/shoot ratios and water-use efficiency. *Journal of Arid Environment*, **60**: 457–481.
- Thapliyal R. 2002. Effect of phosphorus on nitrogen uptake, assimilation, fixation and biomass production in *Albizia lebbeck* Benth. Thesis submitted to the Deemed University Forest Research Institute Dehradun for the degree of Doctor of Philosophy in Forestry, p. 108.
- Uniyal P. 1997. Effect of inoculation and nitrogen fertilizers on nodulation, nitrogen fixation, assimilation and growth behavior in *Dalbergia sissoo* Roxb. Thesis submitted to the Deemed University Forest Research Institute Dehradun for the degree of Doctor of Philosophy in Forestry, p. 142.
- Uniyal P, Pokhriyal TC. 2000. Effect of nitrogen treatment and seasonal variation on biomass production in *Dalbergia sissoo* seedlings. *Indian Journal of Plant Physiology*, **5**(2): 171–173.
- Vasileva V, Kostov O. 2002. Effect of water deficiency stress and N in yield of lucerne (*Medicago sativa* L.). *Ecology and Future*, **1**: 104–107.
- Vasileva V, Kostov O, Vasilev E, Athar M. 2011. Effect of Mineral nitrogen fertilization on growth characteristics of lucerne under induced water deficiency stress. *Pakistan Journal of Botany*, **43**(6): 2925–2928.
- Vessey JK. 2002. Improvement to the inoculation of pea and lentil crops I Southern Manitoba. In: ARDI (Agri Food Research & Development Initiative) Project Results. Manitoba, Canada: Manitoba Agriculture, Food and Rural Initiatives.
- Williams PH, Haynes RJ. 1995. Nitrogen in plant environment. In: Srivastava HS, Singh RP, (eds), *Nitrogen Nutrition in Higher Plants*. New Delhi: Associated Publishing Company, pp. 1–20.
- Xu BC, Shan L, Li FM, Jiang J. 2007. Seasonal and spatial root biomass and water use efficiency of four forage legumes in semiarid northwest China. *African Journal of Biotechnology*, **6**(23): 2708–2714.
- Zhang Z, Fengyan S, Zhenying Z, Jain D. 1990. Studies on inoculation techniques of *Frankia* from *Casuarina cunninghamiana*. In: *Research on Multi-purpose Trees in Asia*, Warnock International, pp. 201–206.